PHOSPHORUS BIOAVAILABILITY AND DIGESTIBILITY IN SOYBEAN MEAL, SPRAY DRIED PLASMA PROTEIN AND MEAT AND BONE MEAL DETERMINED BY DIFFERENT METHODS IN POULTRY

BY

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THESIS

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ABSTRACT

Six experiments were conducted to determine P digestibility or bioavailability for soybean meal (SBM), spray dried plasma protein (SDPP), and meat and bone meal (MBM) using different types of assays in poultry. Experiment 1 evaluated the precision-fed rooster assay and determined the effects of increasing P intakes on excreta P retention values using corn and corn supplemented with increasing amounts of KH_2PO_4 to provide total P intakes of 51-351 mg. The results indicated that excreta P retention values decreased when non-phytate P intakes were 76 mg or higher. Experiment 2 was another precision-fed rooster assay where increasing amounts of SDPP (5-20 g) were fed to provide non-phytate P intakes of 61-242 mg. The results of this experiment were in agreement with the first experiment in that P excretion increased greatly and excreta P retention values decreased as P intake increased. Experiment 3 was a precision-fed rooster assay conducted to evaluate the effect of increasing dietary calcium on excreta P retention values for SBM and SDPP and also the effect of increasing intakes of SDPP and MBM on their excreta P retention values. Excreta P retention values for SBM were 41-42% and excreta P retention values for SDPP again decreased as P intake increased. Dietary calcium level had no significant effect on excreta P retention values for SBM and SDPP. Excreta P retention values for MBM were low (27-35%) at all intakes. The results of these first three experiments suggest that the precision-fed assay may be useful for determining bioavailability of P only if non-phytate P intakes are low and the assay may not be accurate for ingredients which contain high calcium and P levels such as MBM. Experiment 4 was a precision-fed broiler chick assay

conducted to determine ileal P digestibility for SBM, MBM and SDPP. Ileal P digestibility of SBM, MBM, and SDPP was 64, 42, and 94%, respectively. Experiment 5 was an *ad libitum* fed chick assay conducted to determine ileal P digestibility and excreta P retention for SBM, SDPP, and MBM. Chicks were fed diets containing three increasing levels of SBM, SDPP or MBM with the test ingredients providing the only source of dietary P. Three additional diets were used to evaluate a different dietary method where increasing levels of MBM were added to a corn-soybean meal based diet. True ileal P digestibility and true excreta P retention were estimated using regression of ileal P and excreta P output on dietary P content. The results yielded true ileal P digestibility values for SBM, SDPP, MBM (two methods) to be 83, 98, 61, and 23%, respectively. True excreta P retention values for SBM, SDPP, and MBM (two methods) were determined to be 51, 99, 32, and 53%, respectively. Experiment 6 was a chick bone ash bioassay conducted to determine the bioavailability of P in SBM, SDPP, and MBM relative to KH_2PO_4. Chicks were fed a P-deficient cornstarch-dextrose-soybean meal diet supplemented with two increasing levels of P from KH_2PO_4, SBM, SDPP, or MBM. Relative bioavailability values for P in SBM, SDPP, and MBM based on tibia ash and estimated using slope-ratio multiple regression analysis were 36, 125, and 76%, respectively. The results of this study indicated the digestibility/relative bioavailability of the P in SDPP was very high for all methods, but values for SBM and MBM varied greatly among different methods.

To My Best Friend, Denzel

"Do… or do not. There is no try."

-Jedi Master Yoda

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TABLE OF CONTENTS

CHAPTER 1

INTRODUCTION

Phosphorus (P) is an exceedingly important macro-mineral, not only for poultry but for every form of life. In order to secure that an animal's intake is not deficient in this vital macro-mineral, inorganic phosphate is included in diet formulations to ensure that there are no deficiencies, and this inclusion is quite expensive (Li et al., 2016). Although the latter is necessary, the inclusion of inorganic phosphates may not be the most sustainable decision for the future. With increasing dependency on inorganic phosphates, researchers have estimated that phosphorus reserves may be depleted within the next 50-100 years as it is a non-renewable resource (Cordell et al., 2009).

Not only is inorganic P an expensive addition to the feed formulation, there are environmental concerns. Phosphorus runoff is a problem within the agriculture industry as excess P within the manure can lead to an accumulation of P and has the potential to contaminate surrounding bodies of water (Sharpley et al., 1994). There are protocols and procedures that are in place to minimize the risk of this occurring but it is best to decrease overexposure of P at the primary source, which is the feed. It is crucial for the scientific community to be able to assess an accurate percentage of the P that is digestible or available in feed ingredients so that P excretion can be minimized and the animal feed industry can be as efficient as possible to secure the sustainability of our resources for the present and future.

Two ingredients that are important sources of P in poultry diets are soybean meal (SBM) and meat and bone meal (MBM). The first recorded history of the soybean dates back as far as

980 CE, where it was heavily utilized and domesticated in China (Shurtleff and Aoyagi, 2016). China realized the soybean's potential early in history, and as time progressed, so did other nations (Shurtleff and Aoyagi, 2016). Soybeans are currently the most influential oilseed produced in the United States, and they account for about 90% of United States' oilseed industry (USDA, 2017). The soybean contributes two major products within the agriculture industry its oil and meal. Soybean meal is produced through a process called "crushing" where the soybeans are cracked and heated. Once they are cracked, the oil is removed and separated. After the oil is separated, the remaining flakes of the soybean are toasted, dried, and ground to make SBM (NOPA, 2015).

Soybean meal is, debatably, the most utilized source of protein by the poultry and livestock industry (NOPA, 2015). It is an extremely efficient oilseed and its meal contains 44-49% protein as a feed ingredient; it is second to corn as the most planted field crop in the United States (NOPA, 2015; USDA, 2017). Because of the high protein content and production of SBM, it is usually the most cost effective and preferred protein source within broiler diets among poultry feed manufacturers (Sleman et al., 2015). Although the soybean provides a high quality protein source, it does contain anti-nutritional factors that affect its utilization and digestibility. One of these factors is trypsin inhibitors; however, these inhibitors can be destroyed by the heat applied during the production process (Cromwell, 2008). Phytate P is present within soybeans in an indigestible form referred to as phytic acid or phytate (Cromwell, 2008). During the last two decades, high levels of research have resulted in the development of efficacious feed phytases that are routinely added to poultry feeds to convert part of the indigestible phytate into a digestible form of P (Nelson et al., 1968).

As animal production increases to meet human food demand, the amount of waste generated in processing facilities expands as well. A resourceful way of repurposing the disposable characteristics of the animal, such as meat trimmings, parts that are deemed inedible, organs, and condemned carcasses, is to render them and utilize them as an economical feed source for livestock such as meat and bone meal [(MBM) (Miles and Jacob, 2011; Caires et al., 2010)]. This rendered product yields a feed ingredient that is high in crude protein as well as a significant contributor of calcium and P (Miles and Jacob, 2011; Hendriks et al., 2002). Due to these desirable traits, MBM is used to partially replace SBM and inorganic P supplements in poultry diets and is often restricted to less than 5% of the total diet due to its variability in nutrient composition (Miles and Jacob, 2011; Caires et al., 2010). In the future, MBM is expected to continue to be an appropriate and resourceful source of supplemental P for poultry feeds.

Previous research on the availability or digestibility of P in SBM and MBM has yielded highly variable results. It is hypothesized that much of this variability is due to the different types of methods used to determine availability in digestibility. Thus, it is the primary objective of this thesis to determine and compare availability/digestibility values for P in SBM and MBM among several different methods. Spray dried plasma protein will also be evaluated as a positive control ingredient since it has been reported that the P in this ingredient is 100% digestible by swine (Almeida and Stein, 2011).

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CHAPTER 2

THE NUTRITIONAL VALUE OF SOYBEAN MEAL AND MEAT AND BONE MEAL FED TO POULTRY WITH EMPHASIS ON PHOSPHORUS AVAILABILITY OR DIGESTIBILITY: A LITERATURE REVIEW

INTRODUCTION

Since World War II, the United States has come to rely on SBM as a key ingredient of diets within the poultry and swine industry. Soybean meal is a plant-based protein in which both the quality and yield has increased since its introduction to the United States, making it a valuable resource. Soybean meal is a desirable ingredient for many aspects; for one, it is a protein source that is regarded to be highly consistent in quality availability year round (USDA, 2017). Second, SBM is recognized as possessing the highest amount of crude protein among all forms of plant-based proteins, which is desirable for poultry producers as they seek to formulate high energy diets (Waldroup and Smith, 2002). Apparent metabolizable energy (AME) values for SBM have been calculated to be approximately 2,492 kcal/kg dry matter (DM) basis with a net energy (NE) of 1,581 kcal/kg, respectively (Liu et al., 2017). This slightly conflicts with the amount reported within the NRC (1994) that the metaboliazable energy corrected for nitrogen (ME_n) of dehulled SBM is 2,196 kcal/kg DM. However, plenty of variability can occur depending on the source of SBM. Ravindran et al. (2014) reported AME values of SBM ranging from 1,567 to 2,541 kcal/kg for samples collected from the United States (dehulled), Brazil (dehulled), India (nondehulled), and Argentina (dehulled); of these samples India's SBM had the lowest AME.

Soybeans contain approximately 18-21% oil, which is extracted during the production of SBM (Hymowitz et al., 1974). Typically soybean oil is extracted using a hexane solvent, but there are high amounts of energy losses during extraction and drying, resulting in new more

energy-efficient methods currently being developed to extract oil from soybeans (Phan et al., 2009). Soybean oil is an important contributor to many products that are marketed for human consumption, such as their inclusion as the primary oil used in margarine (Erickson, 1995).

Though SBM has many advantages, there are factors that hinder producers from utilizing SBM to its full potential. Because SBM is plant based, about two thirds of its P is bound to phytate (Nelson et al., 1968). Unlike some anti-nutritional factors, phytate is not destroyed when heated during the production process, and it obstructs the absorption of P and other minerals within the intestines (Liener, 2000). As a way to combat this obstacle, there is a large amount of interest in the naturally-occurring enzyme phytase, which is being added to feeds to increase the availability of non-phytate P (Karr-Lilienthal et al., 2005). Phytase supplementation has been shown to improve growth performance of both male and female chickens when fed a plant-based low-P diet (Viveros et al., 2002; Sebastian et al., 1997). In addition to freeing phytate-bound P, phytase may release Ca, Mg, Cu, Zn, Fe, and K by hydrolyzing phytate to reduce Ca-phytate complex formation (Selle et al., 2009).

Another feedstuff that is important within the poultry industry is MBM. This ingredient is a by-product of the meat production industry and may include meat trimmings, inedible parts and organs, certain carcasses, and fetuses of food animals that are often otherwise disposed of in landfills (Miles and Jacob, 2011). This is a resourceful way to repurpose the waste of one industry to be utilized in another, aiding in the reduction of environmental contamination and economic losses. This ingredient is a valuable source of protein, energy, and mineral components. The poultry NRC (1994) reports that MBM has a protein percentage of 50.4, a ME_n of 2,150 kcal/kg and a TME_n of 2,495 kcal/kg. The inclusion of this ingredient not only offers

economic advantages but decreases the industry's dependency on SBM as a highly available source of protein (Ziggers, 2010).

However, considering that MBM is not derived from a single ingredient but is the result of different combinations of ingredients, MBM composition may have a large amount of variation depending on the origin of the sample source (Miles and Jacob, 2011). In a study conducted by Hendriks et al. (2002), 94 commercial MBM samples from 20 different processing plants in New Zealand were evaluated and a large amount of variation was found within several aspects of feed quality. Crude protein percentage ranged from 38.5-73.6% and fat percentage ranged from 2.5-18.5%, with mean values of 56.8 and 10.0%, respectively. The authors concluded that it is difficult to estimate the mean digestibility of this feedstuff as it is too highly variable and there is no way to assess its average nutritional value (Hendriks et al., 2002). Another problem that can occur with MBM is related to how the feedstuff is stored and handled, as prolonged storage can lead the lipid in MBM to become rancid due to oxidative spoilage. This may lead to lower nutritional content and reduced palatability (Miles and Jacob, 2011).

Along with MBM, another blood by-product utilized in the feed industry is spray dried plasma protein (SDPP). Spray dried plasma protein is derived from blood that is collected from the slaughter house industry; plasma within the blood is separated by the use of a centrifuge after an anticoagulant (often sodium citrate) is added. X. The ingredient is then spray dried (Stein, 1996). The completion of this process results in a feedstuff which contains approximately 78% protein (Stein, 1996). The SDPP is used predominately in starter diets for pigs as it has been shown to increase growth performance (Stein, 1996; Kats et al., 1994).

Although SDPP is considered to be a very high quality protein source, it is low in the indispensable amino acids methionine and isoleucine. Peace et al. (2011) conducted a study to

further investigate the biological mechanisms of plasma protein when fed to weaned pigs. The This study suggested dietary inclusion of SDPP for weaned pigs had beneficial effects on the barrier function of the colon as it reduced intestinal mucous; however, these effects were ephemeral unless a high inclusion rate of SDPP was fed. The reason why this occurs is not clear, but their data also showed a reduced fecal score as the inclusion of SDPP was increased, as well as a reduction in intestinal inflammation (Peace et al., 2011).

Effects of processing and type of digestibility assay on amino acid availability in SBM and MBM fed to poultry

Because SBM and MBM are two of the most important sources of protein and amino acids (AA) in poultry feeds, a brief discussion of the factors that affect these characteristics is warranted. According to a survey administered within the poultry industry, results indicate that SBM has an average inclusion rate of 29.4% within starter feeds, 23.8% within grower feeds and 17.8% within finisher diets (Waldroup and Smith, 2002). These values are based on the premise that the soybean meal is not undercooked or overcooked. Lee and Garlich (1992) conducted a study that determined the effects on growth performance of feeding overcooked soybean meal to broilers. They had six diets containing SBM with the SBM decreasing in urease activity of 0.05, 0.03, 0.01, 0.09, 0.00, and 0.00 pH, decreasing trypsin inhibitor activity of 6.10, 5.01, 4.62, 4.83, 2.32, and 1.78 mg/g, and decreasing protein solubility 92, 89, 91, 88, 81, and 81%. The results of this study suggested that there were no differences in SBM AA content among dietary treatments; however, there were significant differences in apparent AA availability for growing chicks. The authors concluded that the retention time or temperature during cooking may be increased by 50% over the normal operating conditions without negatively affecting the AA

availability for SBM; anything above the threshold of 50% would have a negative impact on AA availability and ultimately growth (Lee and Garlich, 1992).

In an effort to better understand the variability of bioavailability of lysine (Lys) and AA quality of MBM, Parsons et al. (1997) conducted several assays to evaluate various samples of MBM. Samples of MBM were obtained from 16 separate suppliers located in United States and Canada and analyzed for various nutrients. A chick assay was also conducted to determine bioavailability of Lys, using a purified crystalline AA diet was used and increasing levels of Lys were added to a basal diet to generate a growth curve. A single level of each MBM sample was added as its own individual dietary treatment, at 10%, in order to provide bioavailable Lys levels that fall within the reference curve. Each diet was fed to chicks from 8-17 days of age. Results of this assay illustrated a linear response in weight gain and gain:feed ratio to Lys supplementation of the basal diet. Lysine bioavailability varied from 43-89% among the 16 MBM sources. Within the same study, Parsons et al. (1997) conducted true digestibility assays using precision-fed cecectomized and conventional roosters where each sample of MBM was fed at 30 g. Results indicated that AA digestibility varied greatly among MBM samples and bird type, but there was no significant interaction between bird type and MBM. Overall, cecectomized birds yielded lower AA digestibility values when compared to conventional birds. The mean true digestibility of the Lys, cysteine (Cys), and methionine (Met) in cecectomized roosters was 81, 58, and 85%, respectively. The largest variability among MBM was observed for Cys where the standard error of the mean was 4.5. The authors concluded that there is a high degree of variation in AA digestibility among commercial sources of MBM; much of this variation was later shown to be due most likely to processing (Parsons et al., 1997).

In order to determine the availability of nutritional components of feed, a wide variety of animal assays have been used, particularly for AA. Three different assays were evaluated by Kim et al. (2012), whose research included SBM and MBM. The three different bioassays that were evaluated were the standardized ileal amino acid digestibility (SIAAD), precision-fed rooster assay (PFR), and precision-fed chick assay (PFC). For the SIAAD experiment, birds were fed diets containing each feedstuff to supply 20% crude protein (CP), and the diets were fed from 16-21 days of age. On Day 21, birds were euthanized and ileal digesta were collected. For the PFR assay, cecectomized roosters were tube-fed 30 g of each feedstuff and excreta were collected 48 hours post-feeding. The last assay that was evaluated was the precision-fed chick assay where 21 day-old broilers were tube-fed the feed ingredients and ileal digesta were collected 4 hours post feeding. This method was developed by Kim et al. (2011) in a previous study where the optimal methods for conducting this assay were determined. The results for AA digestibility varied somewhat among methods, but in most cases, the results were in general agreement. Examples of differences among assays for SBM were for Arg, where values for the PFC and PFR were 93.2 and 88.8%, respectively, and for His where PFC and PFR values were 91.6 and 87.5%, respectively. In contrast, there was no significant difference observed between SIADD values and PFC and PFR values for Arg and His for SBM. Similar results were observed for MBM where there were significant differences among assays for the two indispensable AA, His and Met; however, the differences were not consistent. The AA digestibility values for histidine for the SIAAD and PFR assays were 73.5 and 66.3%, respectively, whereas the AA digestibility values for methionine for SIAAD and PFR were in the opposite direction, 74.0 and 84.9%, respectively. There was no clear explanation for these differences and their inconsistency. The authors concluded that all three assays are acceptable methods for estimating

AA digestibility values as these were only a few significant difference among methods and those differences were not consistent (Kim et al., 2011).

Another study was conducted by Adedokun et al. (2009) where they aimed to determine the effect of bird type on digestibility of AA in several feedstuffs, including SBM and MBM. Broilers, laying hens and cecectomized roosters were used to determine apparent ileal amino acid digestibility (AIAAD) and SIAAD. A basal nitrogen-free diet was used as a negative control to determine endogenous AA loss. Focusing on results of the two dietary treatments for SBM and MBM, diets were formulated to contain approximately 200g/kg of CP and these diets were fed to broilers for 5 days (days 16-21 of age) and laying hens for 5 consecutive days *ad libitum*. In addition, cectomized roosters were precision-fed and excreta were collected 48 hours post-feeding. Laying hens and broiler chickens were euthanized on their fifth consecutive feeding day, and ileal digesta were collected. There was only one significant AIAAD difference between broiler chicks and laying hens fed SBM when compared to roosters, and that was for His. Most or all AIAAD values for broiler chicks were significantly lower when compared with laying hen and rooster values when birds were fed MBM. For SIAAD, values for both SBM and MBM in broiler chicks were significantly lower than values for laying hens and roosters for essential AA. For SBM, laying hen SIAAD values also differed from rooster values for the majority of essential AA. Adedokun et al. (2009) concluded that researchers should be cautious when comparing the above three methods, as obtained digestibility values may differ among methods and vigilance is required when formulating diets using AA digestibility values from more than one method (Adedokun et al., 2009).

Phosphorus content of SBM and MBM

Phosphorus is essential for the formation of eggs and for growth, and is a major component of nucleic acids, phospholipids, and enzymes (Li et al., 2016). The levels of total P and non-phytate P as reported in the NRC (1994) for SBM are 0.62% and 0.22%, respectively. The amount of non-phytate P (0.22%) represents the expected approximate amount of bioavailable P for poultry. Meat and bone meal generally contains approximately 50% crude protein and a minimum of 4% total P, calcium usually does not exceed 2.2 times the level of total P (Miles and Jacob, 2011). Meat and bone meal has a reported percentage of 5.10 total P and a calcium percentage of 10.60 (NRC, 1994).

Availability of P in feed ingredients

The best method to evaluate P bioavailability in feed ingredients has not yet been firmly established. There are a wide range of qualitative and quantitative approaches that have been used to estimate P availability in feed ingredients. Shastak and Rodehutscord (2013) reviewed several of these qualitative (i.e. retention in balance studies, prececal digestibility and comparative whole-body analysis) and quantitative (i.e. bone criteria, blood criteria, growth and feed conversion and combined response criteria) methods. Retention or balance studies aim to estimate the retention of P by measuring the excreta quantitatively or using an indigestible marker and partially sampling the excreta. Alternatively, prececal digestibility assays can be used where the contents of the lower ileum are collected and analyzed for P. The latter has been proposed to be an accurate measurement of P availability in poultry and is not affected by any excess P being excreted in the urine (Shastak and Rodehutscord, 2013). Qualitative approaches often involve a calculation of relative bioavailability where the relationship between P intake and

P excretion from an inorganic source of P is compared to P from the test feed ingredient. Bone criteria often include several measurements, such as bone ash content, bone breaking strength, and bone densitometry, which estimates P availability by observing bone mineralization. Blood criteria assays measure the relationship between dietary P and inorganic P in blood, but these assays have been found to be generally unsuitable assays for estimating P availability (Shastak and Rodehutscord, 2013). When reviewing growth and feed conversion methods, Shastak and Rodehutscord (2013) found conflicting reports about the accuracy of P estimation using these methods, although many believe them to be the most useful at estimating P availability in feed ingredients. However, Shastak and Rodehutscord (2013) concluded that these assays were unsatisfactory. Adeola and Cowieson (2011) also reviewed the growth and feed conversion assay and criticized this method because there are many different factors that affect growth; thus there are a large number of confounding factors affecting the results. In multiple reports, Shastak and Rodehutscord (2013, 2015) encourage the use of prececal digestibility assays when estimating P availability as it uses the regression approach and it seems to be an effective and useful method. Shastak and Rodehutscord (2015) also compared data from qualitative and quantitative criteria to estimate P availability and concluded that it is inappropriate to combine data from both quantitative and qualitative measurements. In order to find the most effective and accurate method, the scientific community should discuss their options and come to an agreement on the best method to estimate P availability in future research (Shastak and Rodehutscord, 2015).

Comparison of Relative Bioavailability Methods to P Digestibility Methods for SBM, MBM, and Spray Dried Plasma Protein

Sullivan (1966) acknowledged the dilemma for determining the availability of P or the value of P for different ingredients for chicks and poults. In an effort to further investigate this obstacle, Sullivan (1966) conducted a study, using two methods, to determine the relative value

of P sources with turkeys fed a corn-soybean meal diet. Each method utilized a combination of body weight gain, percent bone ash, and feed efficiency for estimating availability of P. The first method evaluated 14 different sources of P. These sources were fed at two levels of added P, 0.20 and 0.35%, to day-old poults for four weeks. Results indicated that there was a large variation among sources where percent bone ash ranged from 34.2-44.0%, while body weight gain ranged from 446-619 g. Sullivan (1966) noticed that there was less variation among P sources when P was included at 0.35% compared to 0.20%, which suggested that the P requirement of the poults had been exceeded at 0.35% added P. The second method used male poults, and nine different sources of P were fed at three lower levels of added P (0.16, 0.28, and 0.40%, respectively). Poults were fed the diets for four weeks. One-half of the poults per replication were euthanized to obtain tibia samples after two weeks and the other half of the poults per replicate were fed the diets for 6 weeks and then tibias were collected and weights were recorded. Results of this method showed that the four-week percent bone ash values were less sensitive to differences among P sources than the two-week percent bone ash values. However, when comparing body weight, the two-week body weight data were less sensitive than the four-week body weight data for detecting relative to differences available P values among sources (Sullivan, 1966).

Potter et al. (1995) evaluated seven inorganic sources of P for relative bioavailability using the criteria of body weight and toe ash. The seven sources of P were added to the basal diet to supply inorganic P at six levels of 0.05, 0.08, 0.12, 0.17, 0.23, and 0.32% P, generating 42 diets. Dietary treatments were fed until three weeks of age to male chicks. At that time, the left middle and right middle toe were collected, measured for average percentage ash, and pooled. Results indicated that mortality was higher for diets when the P percentage was low; mortality

was 38% in chicks fed the basal diet plus 0.05% P from inorganic sources (Potter et al., 1995). When the amount of added P was increased, the mortality percentage decreased; when the amount of P added to the diet was 0.05, 0.08, and 0.12%, the mortality was 38, 14.5, and 4.5%, respectively (Potter et al., 1995). Toe ash increased at a decreasing rate when P was added; therefore, significant curvature occurred and made the use of the slope ratio bioassay invalid (Potter et al., 1995). Consequently, a nonlinear regression model was used to estimate relative bioavailability. The average values for relative bioavailability among the seven sources of P were 95.5 and 96.3%, for body weight gain and percentage toe ash, respectively. The authors suggested that these results may support the argument that nonlinear regression models are more useful when estimating relative bioavailability for a wide range of P sources (Potter et al., 1995).

The digestibility methods that have been used to determine P digestibility in feed ingredients for poultry have reported highly variable results. In a recent large study conducted by Rodehutscord et al. (2016), three experimental diets containing increasing inclusion levels of SBM were sent to 17 collaborating stations around the globe. Each station was instructed to use the same diet and determine the prececal P digestibility of SBM. This study resulted in digestibility values that ranged from 19 to 51% (Rodehutscord et al., 2016). Because of this high amount of variability, there are probably several confounding factors that influence P digestibility values. Thus, researchers must use caution when comparing results among labs.

To better understand the content and relative bioavailability of P, Amezcua et al. (2004) conducted several assays using distillers dried grains with solubles (DDGS). Twenty samples of DDGS were retrieved from different ethanol plants located in Minnesota, and were analyzed for DM and total P. The first experiment method consisted of five dietary treatments, where Diet 1 contained 0.1% NPP, Diets 2 and 3 contained an additional 0.05 and 0.1% NPP supplemented

from KH_2PO_4. Diets 4 and 5 contained 12.5 and 25% DDGS, analyzed to contain 0.72% total P. All diets were fed from 8-21 Days of age. This experiment yielded a bioavailable P content of 0.49% and a bioavailability coefficient of 69% for the P in DDGS. The second experiment utilized three samples of DDGS, varying in Lys digestibility, and aimed to determine if P bioavailability was related to Lys digestibility. This experiment consisted of nine dietary treatments, where Diets 1-3 were the same as Diets 1-3 in the first experiment, and Diets 4-9 contained each sample of DDGS supplemented at 8 and 16%. Diets 4-7 contained low digestible Lys DDGS (64 and 61%, respectively), while Diets 8-9 contained high digestible Lys DDGS (79%). Growth performance and bone ash were calculated. The results from this experiment were variable as the P bioavailability coefficient from the DDGS sources ranged from 75-102%, and dietary bioavailable P content ranged from 0.55-0.75%. The source for this variation is unknown; however, the authors suspected that it may be due to the amount of phytin P that is within the different sources of DDGS, or differences in processing of the DDGS (Amezcua et al., 2004).

Amezcua et al. (2007) conducted another study concerning P bioavailability in DDGS as affected by increasing heat during processing and particle size, using several chick experiments. Four DDGS samples were obtained from commercial sources and were analyzed for DM and total P. For all experiments, diets were fed from 8-22 days of age and evaluated via weight gain, feed consumption, feed efficiency, and tibia bone ash. The first experiment had 10 dietary treatments, in which Diet 1 was P deficient, and Diets 2 and 3 contained an additional 0.05 and 0.10% P from KH_2PO_4, respectively. Diets 4-10 contained 10% DDGS processed under different conditions. Diet 4 was conventional DDGS, while Diets 5-7 were DDGS that was autoclaved for 40, 60, and 80 minutes, respectively. Diet 8 was oven-dried DDGS at low temperature (55°C for

3 days) and then autoclaved, Diet 9 was oven-dried at high temperature (121°C for 60 minutes) and Diet 10 was DDGS oven-dried at the low temperature then oven-dried at the high temperature. Results showed that the original conventional DDGS contained 0.76% total P and that the bioavailability of P was increased by most increased heat processing methods (Amezcua et al. 2007). Experiments 2 and 3 were two chick growth assays with seven dietary treatments. Dietary treatments 1-3 were the additional 0.05 and 0.1% P from KH_2PO_4 as described above. In Experiment 2, Diets 4-7 were supplemented with 10% DDGS, which varied in particle size, 837 µm, 573 µm, 631 µm, and 551 µm, respectively. In Experiment 3, Diets 4 and 5 were supplemented with 7 and 14% coarse DDGS, respectively, and Diets 6 and 7 were supplemented with 7 and 14% ground DDGS, respectively. The results showed that there was no significant difference in relative bioavailability of P among DDGS samples varying in particle size from 542 µm vs. 872 µm. The authors concluded that because there were no significant differences due to particle size, therefore, there is no effect of particle size on relative bioavailability of P in DDGS (Amezcua et al., 2007).

In order to determine the true ileal P digestibility of corn and SBM, Mutucumarana et al. (2015b) conducted an experiment comparing two methods, both of which were based on the regression method. One year earlier, this same group had reported a true ileal P digestibility coefficient of SBM to be 0.798 based on a linear regression method which is higher than expected (Mutucumarana et al., 2014). In the Mutucumarana et al. (2015b) study, diets in the first experiment had a 2:1 dietary Ca:P ratio and only one source of protein and P, while the second experiment included dried egg albumen as an additional protein source in the diets, and diets varied in Ca:P ratio. Each experiment consisted of six total test diets, three corn-based diets and three SBM-based diets. In the first experiment, each diet included an increasing

concentration of P where corn Diets 1, 2, and 3 contained 0.52, 1.20, and 1.88 g/kg of P, respectively. The SBM Diets 1, 2, and 3 contained 2.69, 3.43, and 4.16 g/kg of P, respectively. Dietary treatments for the second experiment consisted of corn-based diets containing higher P concentrations of 2.36, 3.04, and 3.72 g/kg in which the Ca:NPP ratios varied from 1.58-2.02:1. Likewise; the SBM-based diets contained 2.71, 3.45, and 4.19 g/kg P in which the Ca:NPP ratios varied from 3.97-4.25:1. Broiler chicks were assigned to each dietary treatment from 21-28 days of age. The birds were euthanized at the end of the experiment and digesta from the ileum were collected and apparent ileal digestibility coefficients (AIDC) were calculated by indicator method. True ileal P digestibility coefficients (TIDC) were then calculated by linear regression. The AIDC for the corn diets in Experiment 1 ranged from 0.403-0.625 and the TIDC was 0.728. In contrast, the AIDC from the second experiment ranged from 0.661-0.882 and the TIDC was 0.426. The majority of the values differed significantly between experiments. The SBM AIDC for Experiment 1 ranged from 0.761-0.807 and the TIDC from regression analysis was 0.740. As observed for corn, Experiment 2 yielded values were lower than Experiment 1 with AIDC values 0.613-0.676 and the TIDC was 0.523. The authors concluded that the two methodologies did in fact influence the estimation of P digestibility. It is not clear what the reason for the differences were. Possible reasons include the protein content of the diets, Ca to P ratio, or the amount of Ca, per se (Mutucumarana et al., 2015b).

Mutucumarana and Ravindran (2016) conducted another study to estimate the true ileal P digestibility of four MBM using what they refer to as the direct method. The use of the direct method means that the only source of dietary P will be the test ingredient. There were five dietary treatments, four of which consisted of varying levels of the four MBM at 38, 60, 60, and 41 g/kg, respectively. The fifth dietary treatment was a P-free diet. Diets were fed *ad libitum*

from 28-31 days of age and then ileal digesta were collected and analyzed for dry matter, Ca, P, and titanium. The AIDC for the P in MBM-1, MBM-2, MBM-3, and MBM-4 were 0.549, 0.526, 0.543, and 0.356, respectively. From feeding the P-free diet, the basal endogenous P loss was determined to be 354 mg/kg of DM intake and this value was used to calculate TIDC for MBM-1, MBM-2, MBM-3 and MBM-4 and resulted in TIDC values of 0.623, 0.617, 0.615, and 0.444, respectively. The authors concluded that the direct method is useful for determining P digestibility in feed ingredients. They also concluded that the P within MBM is not as highly available as the 100% that it is generally regarded to be (Mutucumarana and Ravindran, 2016). This conclusion is supported by a previous study conducted by the same group where true ileal digestibility coefficients of P were 0.693, 0.608, and 0.420 from linear regression analysis for diets formulated to contain three different MBM's at 37.54, 60.17, and 59.8 g/kg (Mutucumarana et al., 2015a).

Van Harn et al. (2017) determined prececal P digestibility of several ingredients, which included porcine bone meal and the inorganic phosphates monocalcium phosphates (MCP) and dicalcium phosphate (DCP). Test diets for porcine bone meal, MCP, and DCP were formulated to contain a Ca:P ratio of 1.2:1.1 and contained 2.22, 2.70, and 2.56 g/kg, respectively, of available phosphorus (AP) on an as-fed basis. Broilers were fed the diets *ad libitum* from 14-24 days of age, and on day 24 birds were euthanized and ileal contents were collected. The prececal AIDC for the diets containing bone meal, MCP, and DCP were 76.5, 81.7, and 78.6, respectively, where the AIDC of the inorganic phosphate diets differed significantly from the bone meal diet. The prececal P AIDC for the ingredients were then calculated by difference where values for bone meal, MCP, and DCP were 78.2, 88.5, and 82.4%, respectively. Values for the bone meal and DCP were not significantly different. The prececal P digestibility values of

bone meal are higher than previous published values for MBM by Mutucumarana et al. (2015a). The authors hypothesize that their higher digestibility values may be due to a lower Ca to P ratio, as there was less Ca to interfere with P utilization. Van Harn et al. (2017) concluded that bone meal may be a viable substitute for inorganic phosphates as a primary source of supplemental P in poultry diets. The latter may allow producers to rely on less inorganic phosphates and aid in a reduction of the use of non-renewable resources, as well as reducing environmental pollution.

To our knowledge, there has not been any research conducted with poultry in reference to P digestibility in SDPP. Therefore, we must refer to research conducted in swine. Almeida and Stein (2011) conducted a study to measure the apparent and standardized total tract digestibility of P in SDPP, avian blood meal and porcine blood meal fed to weanling pigs. Within this study, they had 4 dietary treatments, 6 pigs for each treatment, 3 of the treatments contained each individual type of blood product and the fourth treatment was a P-free diet to measure basal endogenous loss of P. The only source of P within each treatment diet was from the blood products that were used. Within this study, 20% of blood products were included in each diet where SDPP, porcine blood meal, and avian blood meal contained 0.16%, 0.15%, and 0.07% P, respectively. Feces were collected for 5 days to measure apparent total tract digestibility (ATTD) values, which were then corrected for basal endogenous loss to determine the standardized total tract digestibility (STTD) of P (Almeida and Stein, 2011). Results of this study showed that the ATTD for pigs that were fed SDPP had a higher P digestibility of 91.31% compared with either porcine blood meal or avian blood meals which were 76.46 and 57.67%, respectively. When the ATTD was corrected for endogenous P loss to determine the STTD of avian blood meal, porcine blood meal and SDPP, P digestibility values were 86.11, 89.74 and 102.79%, respectively. This evidence suggests that the P in SDPP was completely digested and that the P in the other two

blood meals was also highly digestible. The authors concluded that blood meal and SDPP are excellent sources of highly digestible P and can replace inorganic P. The latter may reduce diet cost and may also reduce excretion of P to lessen the chance of environmental pollution of swine manure (Almeida and Stein, 2011).

CONCLUSION

Among the wide array of assays that have been used for estimating the availability of P in feed ingredients for poultry, there is no true gold standard. Many recent studies are suggesting that prececal digestibility is the best method for determining P digestibility, but the other types of assays have also proven to be useful. The studies and methods reviewed herein show there is no clear answer as to which method is the most accurate as they all have advantages and disadvantages. The current review indicates that there is considerable variability among labs and also that results and P digestibility/availability values from different methods cannot be compared directly with one another. More research is needed to identify the best method that can be applied among labs so that experiments can be replicated and yield consistent values. This exemplifies how imperative it is for the scientific community to come to an agreement as to which method is the most accurate and practical to estimate P availability or digestibility in feed ingredients for poultry and then be able to establish digestible P requirements. The objective of this thesis was to determine P digestibility/availability of SBM, MBM, and SDPP using several different methods. This included evaluating the precision-fed rooster assay which has not previously been evaluated for determining P digestibility or excreta P retention.

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CHAPTER 3

PHOSPHORUS BIOAVIALABILITY IN SOYBEAN MEAL, MEAT AND BONE MEAL, AND SPRAY DRIED PLASMA PROTEIN USING A PRECISION-FED ROOSTER ASSAY

ABSTRACT

Three experiments were conducted to determine phosphorus (P) bioavailability via excreta phosphorus retention values using precision-fed rooster assays for three feed ingredients. The objective of the first rooster assay was to determine the effects of increasing P intakes on excreta P retention values. This assay involved feeding either 20 g of corn, or 20 g of corn supplemented with increasing amounts of KH_2PO_4 to provide total P intakes of 51-351 mg and non-phytate P intakes of 16-316 mg. The results indicated that excreta P retention values decreased when non-phytate P intakes were 76 mg or higher. The second precision-fed rooster assay involved feeding increasing amounts of spray dried plasma protein (SDPP) (5-20 g) which provided non-phytate P intakes of 61-242 mg. The results of this experiment were in agreement with the first experiment in that P excretion increased greatly and excreta P retention values decreased as P intake increased. The results also indicated the excreta P retention value for SDPP was near 100% at the lowest P intake of 61 mg (5g SDPP). The third rooster assay evaluated the effect of increasing dietary calcium (Ca) on excreta P retention values for solvent extracted dehulled soybean meal (SBM) and SDPP, and also the effect of increasing intakes of SDPP and meat and bone meal (MBM) on their excreta P retention values. The specific treatments in the latter precision-fed rooster assay involved feeding either 24 g of SBM, 24 g of SBM + 0.10 g of limestone, or 5, 7.5, or 10 g of SDPP, 5 g SDPP + 0.25 g of limestone, or 1.5, 3, or 10 g of MBM. Dietary Ca level had no significant effect on excreta P retention values for SBM and

SDPP. Excreta P retention values for SBM were 41-42%. Excreta P retention values for SDPP decreased as P intake increased. Excreta P retention values for MBM were low (27-35%) at all intakes. The results of this study suggest that the precision-fed assay may be useful for determining bioavailability of P only if non-phytate P intakes are low and the assay may not be accurate for ingredients which contain high Ca levels such as MBM.

INTRODUCTION

Precision-fed rooster assays have been used to estimate true amino acid digestibility and true metabolizable energy of poultry feed ingredients for decades (Engster et al., 1985; Parsons et al., 1998). However, this assay has not been used to evaluate availability of P in feed ingredients. The precision-fed rooster assay may be an advantageous method to estimate the bioavailability or digestibility of P within feed ingredients because it is faster and less expensive than other common methods and it requires only a small amount of feed. A popular current method among researchers is the use of the prececal digestibility assay in which the data are analyzed through the regression approach (Shastak and Rodehutscord, 2013); however, this method is costly and time consuming. Bone ash or bone P and growth and feed conversion or a combination of these methods have been used to evaluate relative bioavailability of P (Sullivan, 1966; Potter et al., 1995; Kim et al., 2012); however, there are many potential confounding factors within these methods (Shastak and Rodehutscord, 2013). The objective of the current study was to evaluate the precision-fed rooster assay for determining excreta P retention values of three different feed ingredients.

MATERIALS AND METHODS

The protocol for this study was reviewed and approved by the Institutional Animal Care and Use committee at the University of Illinois.

Ingredients and Nutrient Analysis

A sample of solvent-extracted dehulled soybean meal (SBM), two samples of spray dried plasma protein (SDPP1 and SDPP2), and meat and bone meal (MBM) were obtained from commercial sources. The P and Ca levels in the three ingredients were determined using inductively coupled plasma optical emission spectroscopy (Method 985.01 A, B, and D; AOAC International, 2007) after wet washing (Method 975.03 B[b]; AOAC International, 2007). These analyses were conducted at the University of Missouri-Columbia Experiment Station Chemical Laboratory.

Diets and Experimental Design

In Experiment 1, a precision-fed rooster assay was conducted to determine excreta P retention values for diets containing corn or corn supplemented with KH_2PO_4. Twenty-eight Single Comb White Leghorn roosters were housed in individual cages in an environmentally controlled room. They were fasted for 24 hours and then 4 roosters were precision-fed either 20 g of corn, or 20 g of corn supplemented with 0.088, 0.176, 0.264, 0.439, 0.879, or 1.32 g of KH_2PO_4. These dietary treatments were selected to provide a wide range of total P intakes (51-351 mg) and calculated NPP intakes (16-316 mg). Thus, the objective of this experiment was to evaluate the effect of P intake on P retention values because it was expected that the P requirement of the roosters is low. Exceeding the dietary P requirement would be expected to yield reduced P retention values because of increased P excretion in the urine. After precision-feeding, excreta were then collected quantitatively for 48 hours post feeding, freeze-dried,

inspected for any traces of debris, ground and sifted, and analyzed for P. Standardized excreta P retention values were calculated using the values for basal endogenous P losses determined from roosters that had been fasted for 48 hours.

In Experiment 2, a precision-fed rooster assay was conducted to determine excreta P retention values for SDPP1. Sixteen Single Comb White Leghorn roosters were housed in individual cages in an environmentally controlled room. The roosters were fasted for 24 hours and then 4 roosters were precision-fed either 5, 10, 15, or 20 g of SDPP1. These amounts of SDPP1 yielded NPP intakes of 61-242 mg and were selected to again evaluate the effect of NPP intake on excreta P retention values. Excreta were collected quantitatively for 48 hours post feeding, freeze-dried, inspected for any traces of debris, ground and sifted, and analyzed for P. Standardized P retention values were again calculated using the values for basal endogenous P loss determined from roosters that had been fasted for 48 hours.

In Experiment 3, a precision-fed rooster assay was conducted to determine excreta P retention values for SBM, SDPP2, and MBM and the effect of increasing dietary Ca and NPP levels on excreta P retention values. Forty Single Comb White Leghorn roosters were again housed in individual cages in an environmentally controlled room. After being fasted for 24 hours, 4 roosters were precision-fed either 24 g of SBM, 24 g of SBM + 0.10 g of limestone, or 5, 7.5, or 10 g of SDPP2, 5 g SDPP2 + 0.25 g of limestone, or 1.5, 3, or 10 g of MBM. These diets provided a wide range of Ca and NPP intakes and Ca:NPP ratios. Excreta were collected quantitatively for 48 hours post feeding, and processed and analyzed for P as described earlier. Standardized P retention values were again calculated as described earlier.

Statistical Analysis

Data from all three experiments were analyzed using SAS software (SAS Institute. INC., 2010) where the ANOVA procedure was utilized and each individual rooster served as an experimental unit. The least significance difference test was used to assess differences among dietary treatments with significance determined at $P<0.05$.

RESULTS AND DISCUSSION

Nutrient Composition

The analyzed Ca and P content of corn, SDPP, SBM, and MBM are presented in Table 3.1. Corn contained the same amount of Ca that is reported in the NRC (1994) and contained a slightly lower percentage of total P than the NRC (1994) table values, which are 0.02, 0.28, and 0.08% of Ca, total P, and NPP, respectively. The SDPP1 and SDPP2 samples were analyzed to contain 1.21 and 0.98% total P, respectively, which has been reported to be entirely digestible by swine (Almeida and Stein, 2011). Dehulled SBM had analyzed values consistent with those reported in the NRC (1994), which are 0.27, 0.62, and 0.22% Ca, total P, and NPP, respectively. The MBM contained much higher levels of P and Ca, at 9.93 and 4.89%, respectively, which were slightly lower than the table values reported in the NRC (1994) of 10.30 and 5.10% for Ca and P, respectively. A possible reason for the MBM values in the current study being lower than the NRC (1994) table values is that the MBM used in the current study was a pork-derived MBM.

Experiment 1

As inclusion of KH_2PO_4 increased, total P intake increased from 51 to 351 mg and NPP intake increased from 16 to 316 mg (Table 3.2). There was no significant difference in P

excretion as NPP intake increased from 16-56 mg, however, it increased significantly between 76 and 316 mg NPP intake. Likewise, it was observed that decreases in excreta P retention values occurred as NPP intake increased. A large significant decrease in excreta P retention was observed when NPP intake was 76 mg, where 56 NPP mg intake yielded 60% excreta P retention while 76 mg NPP intake yielded only 32% excreta P retention. A possible explanation for this large decrease is that the P requirement of the roosters was exceeded at 76 mg and above, which led to excess absorbed P being excreted in the urine. Thus, since the urine was collected along with the feces (excreta), retention of P would be expected to decrease if the P requirement was exceeded.

Experiment 2

The NPP intake, P excretion, and excreta P retention values for the roosters fed 5, 10, 15, or 20 g of SDPP1 are shown in Table 3.3. The values for NPP intake were calculated by multiplying the amount of SDPP1 intake (mg) by the analyzed percentage of P within the SDPP1. The P excretion increased with increasing NPP intake. Similarly, excreta P retention values decreased from 94 to 60% as NPP intake increased from 61 to 242 mg. Among all dietary treatments, we observed a decrease in excreta P retention values as NPP intake increased ($P < 0.05$). The P in the ingredient, SDPP, was determined to be 100% digestible within swine (Almeida and Stein, 2011). The high excreta P retention value of 94% for the 5 g of SDPP1 treatment in the current study is in agreement with the swine study. The results of this experiment are in general agreement with Experiment 1 where a decrease in excreta P retention was observed as NPP intake increased. This observation again indicates that the P requirement was likely exceeded at SDPP1 intakes of 10 g or greater and that excess P was excreted in the urine. The results of this experiment also suggest that the critical threshold for NPP intake for

SDPP may be higher than that observed for corn + KH_2PO_4 in Experiment 1. The P excretion increased greatly and excreta P retention decreased greatly at NPP intakes of 76 mg or greater in Experiment 1. In contrast, this effect occurred at NPP intakes of greater than 121 mg for SDPP1. Perhaps the P in KH_2PO_4, being inorganic KH_2PO_4 and highly bioavailable, resulted in more rapid P absorption than for the P in SDPP1. Consequently, increased P excretion in the urine may have occurred at a lower NPP intake for corn + KH_2PO_4 than for SDPP1.

Experiment 3

The Ca intake, NPP intake, P excretion, and excreta P retention values for the roosters fed either 24 g of SBM, 24 g of SBM + 0.10 g of limestone, or 5, 7.5, or 10 g of SDPP2, 5 g SDPP2 + 0.25 g of limestone, or 1.5, 3, or 10 g of MBM are shown in Table 3.4. The values for Ca intake were calculated by multiplying the amount of ingredient intake (mg) by the analyzed percentage of Ca. The values for NPP intake were calculated, again by multiplying the amount of ingredient intake (mg) by the analyzed percentage of P within the ingredient. The supplemental limestone levels were selected to provide Ca:NPP levels of 2:1. No significant effect of increased Ca intake on excreta P retention values was observed for SBM and SDPP2, perhaps suggesting that Ca intakes of 98-106 mg do not hinder P bioavailability. As observed in Experiment 2 for SDPP1, P retention values decreased as SDPP2 intakes increased. Excreta P retention for SDPP2 decreased from 79 to 36% as NPP intake increased from 49 to 74 mg. The latter results are in general agreement with Experiment 1 where excreta P retention decreased from 60 to 32% when NPP intake increased from 56 to 76 mg. These overall results suggest that the critical upper P intake level may be between 50 from 75 mg for the precision-fed rooster assay. Phosphorus intakes above the latter may exceed the P requirement of the roosters, possibly resulting in

increased P excretion in the urine and reduced excreta P retention values. For MBM, as NPP intake increased, P excretion increased greatly as well. However, the excreta P retention values did not differ significantly among dietary MBM and P intakes.

When comparing the results for the current study with those of previous studies for SBM, SDPP, and MBM, some results are in agreement and some are not. For SBM, the rooster assay yielded excreta P retention values of 41 and 42%. These values seem reasonable and are in general agreement with the NRC (1994), where the total P of SBM is listed at 0.62% and the NPP is 0.22%. Dividing the NPP by the total P value yields an estimate of 35% availability. Likewise, in the NRC (2012), the total P of SBM is listed as 0.71% and the phytate P is 0.38%. Thus, dividing the NPP by the total P yields an availability estimate of 46%. These values are also in general agreement with the relative bioavailability value of 34% reported by Karr-Lilienthal et al. (2005), for SBM extracted for 45 minutes. The latter value was based on bone ash. In a more recent study conducted by Hanna et al. (2017), relative bioavailability values of P in SBM were reported to be 34-45%, also based on bone ash.

In contrast, the above values of 34 to 45% are generally lower than ileal P digestibility and excreta P retention values reported for SBM in balance studies. For example, Nwokolo et al. (1976) reported an excreta P retention value of 89% for SBM. Liu et al. (2012) reported that the determined standardized P retention values of a SBM diet in two separate experiments were 50.5 and 52.9%. The true ileal P digestibility value of SBM was reported to be 80% by Mutucumarana et al. (2014). Slightly later, Mutucumarana et al. (2015) reported true ileal P digestibility values to be 74 and 52% for SBM using two different methods. In a digestibility ring test (Rodehutscord et al. 2016) with 17 collaborating stations, the mean apparent prececal digestibility of P in SBM diets was 61% and prececal P digestibility estimated from regression

analysis varied between 19 and 51%, suggesting high variability among laboratories. In a study conducted using the regression method to estimate true P digestibility, Dilger and Adeola (2006) concluded that the regression approach is an appropriate method to estimate endogenous P loss in broilers and that true prececal P digestibility in conventional SBM was 94% and true excreta P retention in conventional SBM was 60%. In a more recent study, Liu et al. (2013) found that the true prececal digestibility of P in SBM was 71, 46, and 51% when the Ca:P ratio was increased from 0.8, 1.6, and 2.0, respectively, using the regression method.

For SDPP, the results of the current study indicated that, based on excreta P retention values at a low SDPP intake, the availability of the P was high (79-98%). As mentioned earlier, these results are in general agreement with those of Almeida and Stein (2011) who reported that P in SDPP was 100% digestible. Thus, the P in SDPP is highly available for both poultry and swine.

For MBM, the rooster assay used herein yielded low excreta P retention values (27-32%). These values are much lower than expected since the P in MBM and other animal meals is generally assumed to be highly available and well utilized (NRC, 1994). Previous studies determining relative bioavailability values for P in animal meals such as MBM and fish meal (based primarily on bone ash measurements) concluded that the P in these animal meals was 100% available (Waldroup et al., 1965; Spandorf and Leong, 1965; Sell and Jeffrey, 1996). In contrast, a more recent study using a balance method to determine ileal P digestibility values for three MBM sources yielded much lower estimates for P availability (Mutucumarana et al., 2015). In that study, true ileal digestibility of P in the three MBM was 69, 61 and 42%. As mentioned above, the excreta P retention values determined herein using a rooster balance assay were also low. The reason why the balance type studies are yielding low estimates of P availability for

MBM is unknown. The very low excreta P retention values obtained with the rooster assay may possibly be due to the high Ca level in MBM and may indicate that the rooster assay is not suitable for determining P bioavailability in animal meals that contain a high Ca level.

In summary, it seems that the precision-fed rooster assay may be able to provide approximate estimates of P availability in plant feed ingredients if the NPP or available P intake is low, such as between 50 and 60 mg. If the NPP intake exceeds those levels, excreta P retention values will likely be underestimated because excess P may be excreted in the urine. The need to maintain a low NPP intake of 50 to 60 mg is a challenge because those P intakes are much lower than the endogenous P excretion. The endogenous P excretion (measured with fasted roosters) was 107 to 119 mg during the 48-hour excreta collection period in the current study. Ideally, in a nutrient digestibility or retention assay, it is desirable that the endogenous excretion of the test nutrient be small compared to the intake level of the nutrient. Otherwise, any variation or error in measuring or estimating endogenous P excretion may have a profound effect on the excreta retention value. Consequently, the need to have a low NPP or bioavailable P intake and the high endogenous P excretion relative to P intake indicates that the precision-fed rooster assay may not be highly useful or practical for routine evaluation of P availability in feed ingredients for poultry.

TABLES

Table 3.1. Analysis of Ca and total P and calculated non-phytate P content of feed ingredients.

Ingredient	Ca (%)	Total P (%)	NPP[1,2] (%)
Corn	0.02	0.26	0.08
Spray dried plasma protein 1	0.12	1.21	1.21
Spray dried plasma protein 2	0.10	0.98	0.98
Dehulled soybean meal	0.28	0.59	0.22
Meat and bone meal	9.93	4.89	4.89

[1]NPP indicates non-phytate P.

[2] Non-phytate P calculated using the NRC (1994) table value for NPP content of corn and soybean meal.

Table 3.2. Phosphorus excretion and excreta P retention values for roosters in Experiment 1.[1]

Dietary treatment	Total P intake (mg)	NPP[2] intake (mg)	P excretion (mg)	P retention (%)
1. 20 g corn	51	16	120[d]	75[a]
2. 20 g corn + 0.088 g KH_2PO_4	71	36	132[d]	66[a]
3. 20 g corn + 0.176 g KH_2PO_4	91	56	145[d]	60[a]
4. 20 g corn + 0.264 g KH_2PO_4	111	76	183[c]	32[b]
5. 20 g corn + 0.439 g KH_2PO_4	150	116	213[c]	30[b]
6. 20 g corn + 0.879 g KH_2PO_4	251	216	309[b]	20[b]
7. 20 g corn + 1.320 g KH_2PO_4	351	316	348[a]	31[b]
Pooled SEM			11.6	8.8

[a-d] Means within a column with no common superscript differ significantly ($P<0.05$).

[1]Values are means of four individually-caged roosters.

[2]NPP indicates non-phytate P.

Table 3.3. Phosphorus excretion and excreta P retention values for roosters fed spray dried plasma protein sample two (SDPP1) in Experiment 2.[1]

Dietary treatment	NPP[2] intake (mg)	P excretion (mg)	P retention (%)
1. 5 g SDPP1	61	119[c]	94[a]
2. 10 g SDPP1	121	139[c]	83[ab]
3. 15 g SDPP1	182	176[b]	69[bc]
4. 20 g SDPP1	242	217[a]	60[c]
Pooled SEM		6.8	5.7

[a-c] Means within a column with no common superscript differ significantly (P<0.05).

[1]Values are means of four individually-caged roosters.

[2]NPP indicates non-phytate P.

Table 3.4. Phosphorus excretion and excreta P retention values for roosters in Experiment 3.[1]

Dietary treatment	Ca intake (mg)	NPP[2] intake (mg)	P excretion (mg)	P retention (%)
1. 24 g soybean meal	67	53	203^{bc}	41^{b}
2. 24 g soybean meal + 0.10 g limestone	106	53	200^{bc}	42^{b}
3. 5 g SDPP2	5	49	128^{e}	79^{a}
4. 5 g SDPP2 + 0.244 g limestone	98	49	137de	62^{a}
5. 7.5 g SDPP2	8	74	166^{cde}	36^{b}
6. 10 g SDPP2	10	98	178^{cd}	40^{b}
7. 1.5 g meat and bone meal	149	73	166^{cde}	35^{b}
8. 3 g meat and bone meal	298	147	225^{b}	27^{b}
9. 10 g meat and bone meal	993	489	450^{a}	32^{b}
Pooled SEM			15.4	6.6

[a-e] Means within a column with no common superscript differ significantly ($P<0.05$).

[1]Values are means of four individually-caged roosters.

[2]NPP indicates non-phytate P; SDPP2 indicates spray dried plasma protein sample two.

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CHAPTER 4

PHOSPHORUS DIGESTIBILITY AND BIOAVAILABILITY IN SOYBEAN MEAL, MEAT AND BONE MEAL, AND SPRAY DRIED PLASMA PROTEIN DETERMINED USING DIFFERENT METHODS

ABSTRACT

Three experiments were conducted to determine phosphorus (P) bioavailability using several methods. The objective of the first experiment was to determine ileal P digestibility of soybean meal (SBM), meat and bone meal (MBM), and spray dried plasma protein (SDPP) using a precision-fed broiler chick assay. This assay involved feeding 8 g of SBM, MBM, or SDPP to broiler chicks at 21 days of age. At six hours after feeding, ileal digesta were collected. Ileal P digestibility of SBM, MBM, and SDPP2 was 64, 42, and 94%, respectively. The objective of the second experiment was to determine ileal P digestibility and excreta P retention of SBM, SDPP, and MBM using an ad-libitum fed chick assay. On Day 17 of age, chicks were placed on one of twelve dietary treatments; Diets 1-3 contained increasing levels of 18-54% SBM, Diets 4-6 contained 7-21% SDPP2, Diets 7-9 contained 2.5-7.5% MBM, with the ingredients providing the only source of dietary P. Diets 10-12 used a different dietary approach with increasing levels of MBM 0.0, 2.0, and 4.0%, respectively, being added to a corn-soybean meal based diet. On Day 21, ileal digesta and excreta were collected. True ileal P digestibility and true excreta P retention were estimated using regression of ileal P and excreta P output on dietary P content. The results yielded true ileal P digestibility values for SBM, SDPP, MBM (two methods) to be 83, 98, 61, and 23%, respectively. True excreta P retention values for SBM, SDPP, and MBM (two methods) were determined to be 51, 99, 32, and 53%, respectively. The objective of the third experiment was to determine the bioavailability of P in SBM, SDPP, and MBM relative to KH_2PO_4 using a chick bone ash bioassay. The 11 dietary treatments included a P-deficient

cornstarch-dextrose-soybean meal diet supplemented with 0.05 and 0.10% P from KH_2PO_4, with and without added Ca, 12.5 and 25.0% SBM, 5.0 and 10.0% SDPP, or 1 and 2% MBM, respectively. Bioavailability of P based on tibia ash and estimated using the multiple-regression slope-ratio method was 36, 125, and 76% for SBM, SDPP, and MBM, respectively, relative to KH_2PO_4. The results of this study indicated the digestibility/relative bioavailability of the P in SDPP was very high for all methods, but values for SBM and MBM varied greatly among different methods.

INTRODUCTION

It is among debate within the scientific community as to which method is most effective and accurate for determining P digestibility and bioavailability for poultry. Shastak and Rodehutscord (2013) evaluated several methods for determining P availability in broilers and concluded that prececal digestibility may be the most appropriate method for evaluating P in poultry. Mutucumarana et al. (2015a) used this method to estimate true ileal P in MBM. By regressing P output against dietary P content, the results of this experiment yielded true ileal P digestibility coefficients for three sources of MBM that were 0.693, 0.608, and 0.420. The authors concluded that the reason for the variable and lower-than-expected values were unclear and that more research was necessary (Mutucumarana et al., 2015a). Within the same year, Mutucumarana et al. (2015b) measured true ileal P digestibility of SBM using two different methods. The first method used the test ingredient as the only source of protein, P, and Ca, and the second method used dried egg albumen as an additional protein supplement along with the test SBM. True ileal P digestibility coefficients were again determined by regressing P output in ileal digesta against dietary P content. The true ileal P digestibility coefficients for SBM were determined to be 0.740 and 0.523 for method 1 and method 2, respectively. The authors

concluded that the lower digestibility coefficient for SBM in method 2 may indicate that ileal P digestibility may be influenced by dietary Ca:total P ratios, although more research is necessary to also understand if the dietary inclusion of dried albumen influenced P digestibility (Mutucumarana et al., 2015b). The results of the above ileal digestibility studies indicated that the digestibility of P in SBM is equal to or greater than that for MBM, which is unexpected.

Kim et al. (2011) developed a precision-fed chick ileal broiler assay to determine amino acid (AA) digestibility using ileal digesta from 21-day-old broiler chicks. This assay consists of precision-feeding chicks 6-10 grams of a test ingredient and collection of ileal digesta approximately 6 hours post feeding. The results of that study concluded that this assay may be useful for determining AA digestibility in chicks. The precision-fed chick assay may also be accurate and useful for determining ileal digestibility of P in feed ingredients for poultry. No research has been published in scientific journals on studies to evaluate the latter.

Another method that has been used often to evaluate P bioavailability in feed ingredients is the relative bioavailability method in which bioavailability is determined by comparing bone ash responses for feed ingredients compared with a highly available P reference standard such as potassium phosphate or dicalcium phosphate. Using this type of method, Sands et al. (2003), Karr-Lilienthal et al. (2005), and Hanna et al. (2017) all reported that the relative bioavailability of P in SBM is approximately 35%. For MBM, the bioavailability of P has been reported to be approximately 100% when using the relative bioavailability method (Waldroup et al., 1965; Sell and Jeffrey, 1996). Thus, the results that have been obtained using the relative bioavailability method for SBM and MBM seem to differ greatly from those obtained by the ileal on prececal digestibility method discussed above. One potential reason for part of the variation among studies is that different samples of SBM and MBM were evaluated in the different studies.

Therefore, the objective of the current study was to determine ileal P digestibility, excreta P retention and relative P bioavailability in the same sample of SBM and MBM. In addition, spray dried plasma protein (SDPP) was also evaluated as a high protein ingredient that was expected to have high P digestibility based on research published in swine (Almeida and Stein, 2011).

MATERIALS AND METHODS

The protocol for this study was reviewed and approved by the Institutional Animal Care and Use committee at the University of Illinois.

Ingredients and Nutrient Analysis

Samples of dehulled solvent-extracted soybean meal (SBM), spray dried plasma protein (SPDD), and meat and bone meal (MBM) were obtained from commercial sources. The SPDD sample was the same as the SDPP2 that was evaluated in Chapter 3. Ingredients were analyzed for dry matter (Method 930.15; AOAC International, 2007), crude protein (Method 990.03; AOAC International, 2007), crude fat (Method 920.93 (A); AOAC International, 2007), crude fiber (Method 978.10; AOAC International, 2007), and ash (Method 942.05; AOAC International, 2007). The P and Ca levels in the three ingredients were determined using inductively coupled plasma optical emission spectroscopy (Method 985.01 A, B, and D; AOAC International, 2007) after wet washing (Method 975.03 B[b]; AOAC International, 2007). These analyses were conducted at the University of Missouri-Columbia Experiment Station Chemical Laboratory.

Diets and Experimental Design

Chicks in all experiments were housed in batteries with raised wire floors in an environmentally-controlled room. In Experiment 1, SBM, MBM, and SDPP were evaluated to determine ileal P digestibility using a precision-fed chick assay. Ross 308 broiler chicks were fed

a nutritionally-complete corn-SBM starter diet until 20 days of age. On day 20 of age, chicks were fasted overnight and were then precision-fed 8 grams of SBM, MBM, or SDPP. The chicks were then placed in a starter battery where water was available *ad libitum*. There were 4 pens of 4 chicks per dietary treatment. All dietary treatments contained 0.5% TiO_2 as an indigestible marker. At six hours post precision-feeding, chicks were euthanized using CO_2, and ileal digesta were collected from Meckel's diverticulum to the ileo-cecal junction. Apparent ileal P digestibility was then calculated.

In Experiment 2, SBM, SDPP, and MBM were evaluated to determine ileal P digestibility and excreta P retention using *ad libitum* fed chicks. The first nine diets were dextrose-based and contained the test ingredients as the only source of dietary P (Table 4.1). Diets 1-3 contained increasing levels of 18, 36, and 54% SBM, respectively. Diets 4-6 contained increasing levels of 7, 14, and 21% SDPP2, respectively. Diets 7-9 contained increasing levels of 2.5, 5.0, and 7.5% MBM, respectively. Diets 10-12 were formulated to evaluate ileal P digestibility and excreta P retention for MBM using a different dietary approach. Increasing levels of 0.0, 2.0, and 4.0% MBM were added to a corn-SBM-potato protein based basal diet (Table 4.2) following the general method of Rodehutscord (2013). Calculated nonphytate P levels in all diets were maintained at 0.30% or less to be well below the nonphytate P requirement of the chicks (NRC, 1994). Ross 308 broiler chicks were fed a nutritionally-complete corn-SBM starter diet until 16 days of age. On Day 16 of age, chicks were fasted overnight prior to being placed on experiment. On Day 17, chicks were weighed, wing banded, and allotted to the 12 dietary treatments, described above, through a complete randomized design so that mean body weight was similar across dietary treatments. There were 5 chicks per pen and 5 pens per dietary treatment. From 17-21 days of age, experimental diets and water were available for *ad libitum* consumption. On

day 21, feed intakes per pen and body weight of each chick were recorded. The chicks were then euthanized using CO_2 gas and ileal digesta were collected. On 20 and 21 days of age, excreta were collected. Ileal digesta and excreta were then freeze-dried and analyzed for P. Apparent Ileal P digestibility and excreta P retention were then calculated. All diets contained 0.5% titanium dioxide as a digesta marker.

In Experiment 3, SBM, MBM, and SDPP were evaluated for relative P bioavailability using a chick bone ash assay. A P-deficient cornstarch-dextrose-SBM diet was fed as Diet 1 (Table 4.3). Diets 2 and 3 contained 0.05 and 0.10% added P from potassium phosphate, respectively. Diets 4 and 5 contained 0.05 and 0.10% added P from potassium phosphate but also contained 0.10 and 0.20% added calcium from limestone, respectively, to provide Ca and P in a 2:1 ratio. The latter two diets were included to provide a reference standard for MBM since this ingredient contains a large amount of Ca in an approximate 2:1 ratio to P. Diets 6 and 7 contained 12.5 and 25.0% SBM, respectively. Diets 8 and 9 contained 5.0 and 10.0% SDPP, respectively. Diets 10 and 11 contained 1 and 2% MBM, respectively. All ingredients were substituted in place of dextrose and cornstarch. New Hampshire x Columbian male chicks were fed a nutritionally-complete starter diet for seven days. On Day 7 of age, chicks were fasted overnight prior to being placed on experiment. On Day 8 of age, chicks were weighed, wing banded, and allotted to the 11 dietary treatments through a complete randomized design so that mean body weight was similar across treatments. There were 5 chicks per pen and 5 pens per dietary treatment. Chicks were on trial from 8 to 22 days of age, experimental diets and water were available *ad libitum*. On Day 22 of age, feed intake per pen and final body weight of each chick were recorded. Then, using CO_2, chicks were euthanized and the right tibia bone was collected, autoclaved, cleaned and dry-ashed at 600° C in a muffle furnace.

Statistical Analysis

For Experiments 1, 2, and 3, data for ileal P digestibility, excreta P retention, weight gain, feed intake, gain to feed ratio and bone ash were analyzed using the PROC ANOVA procedure of SAS with pen as the experimental unit (SAS Institute., 2010). Differences among treatment means were assessed using the least significant difference test. For Experiment 2, simple linear regression analyses were used to regress ileal digesta or excreta P output (g/kg DM) on dietary P content (g/kg) for SBM, SDPP, and MBM treatments. Thus, the slope value from the regression equation represents indigestible P. This slope value was subtracted from one to obtain the ileal P digestibility and excreta P retention coefficients. For Experiment 3, multiple linear regression was conducted by regressing tibia ash (mg/tibia) on supplemental P intake (mg/chick) from potassium phosphate, SBM, SDPP, or MBM using the GLM procedure of (SAS Institute. 2010). The slope-ratio method was then used to estimate the bioavailability of P in SBM and SDPP, relative to potassium phosphate alone. The bioavailability of P in MBM was calculated relative to the potassium phosphate plus Ca treatments. Significance was assessed at $P<0.05$.

RESULTS AND DISCUSSION

Nutrient Composition

The analyzed nutrient composition of SBM, SDPP2, and MBM are shown in Table 4.5. The CP in SBM was higher than the value of 48.5% reported in the NRC (1994), whereas the MBM was lower than the reported value of 50.4% in the NRC (1994). The greatest analyzed value of CP was for SDPP and was similar to the reported value in the NRC (2012) of 77.84%.

The analyzed Ca and total P for SBM were 0.28 and 0.59%, respectively, which are in agreement with those reported in the NRC (1994). The MBM used in this study was derived from pork sources, the values of 10.94% for Ca and 5.26% for P from the NRC (2012) are slightly higher than the analyzed values for the MBM evaluated herein. The analyzed Ca and P values for SDPP were 0.10 and 0.98%, respectively, which are slightly lower than those reported in the NRC (2012).

Experiment 1

Ileal P digestibility values for P in SBM, MBM, and SDPP determined using the precision-fed chick assay are located in Table 4.6. There were significant differences in ileal P digestibility among dietary treatments with values varying from 42-94%. The highest percentage of ileal P digestibility was 94 for SDPP which is in agreement with a previous study from Almeida and Stein (2011) stating that SDPP is a highly digestible source of P for swine. Almeida and Stein (2011) reported that pigs fed SDPP had a standardized total tract digestibility (STTD) of P that was 102.8%. Soybean meal resulted in the second highest percentage of ileal P digestibility at 64. These results are in general agreement with values reported from Dilger and Adeola (2006) for SBM (60%) and Mutucumarana et al. (2015b) who reported true ileal P digestibility of SBM to be 72%. The MBM herein yielded the lowest ileal P digestibility value at 42%, contradicting previously published articles that concluded the P in animal products are highly or totally available for poultry (Waldroup et al., 1965; Sell and Jeffrey, 1996; NRC, 1994). Mutucumarana et al. (2015a) reported true ileal P digestibility of MBM to vary from 42 to 69%. Thus, the results of the current study and the Mutucumarana et al. (2015a) study suggests

that SBM, a plant based feed ingredient, has P digestibility equal to or higher than that of MBM, an animal by-product, when using a prececal or ileal P digestibility assay.

Experiment 2

The ileal P digestibility and excreta P retention values for the 12 dietary treatments are shown in Table 4.7. For Diets 1-9 in which the test ingredients were the only source of dietary P, apparent ileal P digestibility for SDPP was high, ranging from 80 to 94%, and apparent excreta P retention was also high, ranging from 74 to 93%. These results are in general agreement with Experiment 1 suggesting that SDPP is a highly digestible ingredient for P. In agreement with Experiment 1 apparent ileal P digestibility values for SBM in Diets 1-3 were higher (74-85%) than those of MBM in Diets 7-9 (66-79%). Soybean meal, Diets 1-3 (49-63%), also resulted in greater excreta P retention when compared to that of MBM, Diets 7-9 (32-39%). The current apparent ileal P digestibility values of 66-79% are slightly higher than values from Mutucumarana et al. (2015a) reporting apparent ileal P digestibility values of three samples of MBM ranging from 49 to 69%. This study by Mutucumarana et al. (2015a) also used the linear regression approach where true ileal digestibility values were determined by regressing P output against dietary P content; the values ranged from 42 to 69%.

True ileal digestibility values determined by using the linear regression between ileal or excreta P outputs vs. dietary P content were also determined in the current study and are presented in Table 4.8. These results yielded true ileal digestibility and true excreta retention coefficients that were lower for MBM than for SBM, again, contradicting the earlier studies which indicated P from animal sources are highly or totally available (Waldroup et al., 1965; Sell and Jeffrey, 1996). For SDPP, true ileal P digestibility and true excreta P retention coefficients

were very high at 98 and 99%, respectively, which are in agreement with the study of Almeida and Stein (2011) which indicated that the P in SDPP is 100% digestible. The low R^2 values for the SDPP regression were due to the very low amount of P output in the ileal digesta and because of the very high P digestibility.

As mentioned above, dietary treatments 10-12 were formulated similar to the protocol suggested by Rodehutscord (2013) where the ileal digestibility of P and excreta retention of P in the MBM was determined when adding increasing levels of the MBM to a corn-SBM based diet. A corn-SBM-potato protein diet was used in the current study. Mutucumarana et al. (2015b) used this method by adding dried egg albumen to supplement protein as well as a small amount of dietary Ca and P, and the true ileal digestibility coefficients were calculated by regressing P output in ileal digesta against dietary P content. Mutucumarana et al. (2015b) found the true ileal P digestibility coefficient of SBM using this method was 0.52 which differed from the value obtained when SBM was the only source of dietary P. Employing this type of method in Diets 10-12 of the current study, inclusion of 2 or 4% MBM resulted in decreased ileal digestibility of P compared to the unsupplemented diet (Diet 10) whereas no significant effect of increasing MBM inclusion was observed for excreta P retention values (Table 4.7). When P output in ileal digesta and excreta were regressed on dietary P content, true ileal P digestibility and excreta P retention values were 23.3 and 52.7%, respectively (Table 4.8). As discussed above, these values are much lower than the values of approximately 100% that have been reported in several earlier studies or publications (Waldroup et al., 1965; Sell and Jeffrey, 1996; NRC, 1994).

Experiment 3

In the relative P bioavailability chick assay (Experiment 3), weight gain was generally significantly increased ($P < 0.05$) by the highest level of supplemental P from potassium

phosphate, SDPP and MBM (Table 4.9). Feed intake and gain:feed responses to supplemental potassium phosphate, SBM, SDPP and MBM were not consistent. A linear response in bone ash was observed among treatments as diets increased in supplemental P, with the largest response being observed for SDPP. Also, as Ca from limestone was supplemented along with potassium phosphate (Diets 4 and 5), bone ash decreased numerically or significantly ($P<0.05$) compared with potassium phosphate supplemented alone, which suggests that the added Ca affected the utilization of P from the ingredient.

Calculated relative bioavailability values from the multiple regression analysis are presented in Table 4.10. The high relative bioavailability value for P in SDPP (the calculated value exceeded 100%) is in agreement with previous Experiments 1 and 2 and further indicated that it is a highly digestible or available source of P. Relative bioavailability values for P in SBM and MBM, however, do not agree with previous Experiments 1 and 2, with the results of Experiment 3 indicating the P in MBM is more highly available than the P in SBM. The relative bioavailability value of 36% for SBM is in excellent agreement with the values of 39% reported by Hanna et al. (2017) and 36% reported by Sands et al. (2003) when using the same type of chick bone ash method. The results that the P in MBM was more highly available than the P in SBM seem reasonable considering previous research has stated that MBM is a highly available source of P (Waldroup et al., 1965; Sell and Jeffrey, 1996), and the NRC (1994) states that the P in animal products is generally considered to be well utilized. Considering that SBM is a plant-based source of P and a large amount of the P is present as phytate P, which is poorly digested (NRC, 1994), it is expected that an animal-based source of P is more highly available than that of a plant-based source of P such as SBM. In contrast, the results of the ileal digestibility and excreta P retention values in Experiments 1 and 2 in the current study and previous such studies

by Nwokolo et al. (1976), Dilger and Adeola (2006), Mutucumarana et al. (2014), Mutucumarana et al. (2015a), and Mutucumarana et al. (2015b), indicated that the ileal digestibility and/or excreta P retention of the P in SBM was equal to or greater than MBM. The reason for discrepancy between results obtained using a relative bioavailability chick assay and the results obtained with ileal digestibility or excreta retention balance assays are unknown. It is also unknown as to why the balance-type assays are yielding values that are seemingly too high for SBM and too low for MBM. More research is definitely needed in the latter two areas.

TABLES

Table 4.1. Ingredient composition of experimental Diets 1-6 in Experiment 2.

	Dietary treatments					
	Soybean meal			Spray dried plasma protein		
Ingredient,%	1	2	3	4	5	6
Dextrose	63.575	45.50	27.43	74.31	66.96	59.62
Cornstarch	10.00	10.00	10.00	10.00	10.00	10.00
Meat and bone meal	-	-	-	-	-	-
Corn	-	-	-	-	-	-
Soybean meal	18.00	36.00	54.00	-	-	-
Spray dried plasma protein	-	-	-	7.00	14.00	21.00
Soybean oil	2.00	2.00	2.00	2.00	2.00	2.00
Limestone	0.075	0.15	0.22	0.34	0.69	1.03
Solka floc	5.00	5.00	5.00	5.00	5.00	5.00
Salt	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin mix[1]	0.20	0.20	0.20	0.20	0.20	0.20

Table 4.1. cont.

Mineral mix[2]	0.15	0.15	0.15	0.15	0.15	0.15
Choline chloride	0.10	0.10	0.10	0.10	0.10	0.10
TiO_2	0.50	0.50	0.50	0.50	0.50	0.50
Calculated analysis:						
Ca	0.08	0.16	0.24	0.14	0.28	0.41
Nonphytate P	0.04	0.08	0.12	0.07	0.14	0.21

[1]Provided per kilogram of diet: retinyl acetate, 4,400 IU; cholecalciferol, 25µg; DL-α-tocopheryl acetate, 11 IU; vitamin B_{12}, 0.01 mg; riboflavin, 4.41 mg; D-pantothenic acid, 10 mg; niacin, 22 mg; menadione sodium bisulfite, 2.33 mg.

[2]Provided as milligrams per kilogram of diet: manganese, 75 from $MnSO_4 \cdot H_2O$; iron, 75 from $FeSO_4 \cdot H_2O$; zinc, 75 mg from ZnO; copper, 5 mg from $CuSO_4 \cdot 5H_2O$; iodine, 75 from ethylene diamine dihydroiodide; selenium, 0.1 from $NaSeO_3$.

Table 4.2. Ingredient composition of experimental Diets 7-12 in Experiment 2.

	Dietary treatments					
	Meat and bone meal			Meat and bone meal		
Ingredient,%	7	8	9	10	11	12
Dextrose	79.34	77.39	75.45	-	-	-
Cornstarch	10.00	10.00	10.00	15.37	13.39	11.39
Meat and bone meal	2.00	4.00	6.00	-	2.00	4.00
Corn	-	-	-	51.00	51.00	51.00
Soybean meal	-	-	-	20.00	20.00	20.00
Spray dried plasma protein	-	-	-	-	-	-
Potato protein	-	-	-	10.00	10.00	10.00
Soybean oil	2.00	2.00	2.00	2.00	2.00	2.00
Limestone	-	-	-	0.28	0.26	0.26
Solka floc	5.00	5.00	5.00	-	-	-
Salt	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin mix[1]	0.20	0.20	0.20	0.20	0.20	0.20
Mineral mix[2]	0.15	0.15	0.15	0.15	0.15	0.15

Table 4.2. cont.

Choline chloride	0.10	0.10	0.10	0.10	0.10	0.10
KH_2PO_4	0.31	0.26	0.20	-	-	-
TiO_2	0.50	0.50	0.50	0.50	0.50	0.50
Calculated analysis:						
Ca	0.20	0.40	0.60	0.17	0.36	0.56
Nonphytate P	0.10	0.20	0.30	0.085	0.18	0.28

[1]Provided per kilogram of diet: retinyl acetate, 4,400 IU; cholecalciferol, 25µg; DL-α-tocopheryl acetate, 11 IU; vitamin B_{12}, 0.01 mg; riboflavin, 4.41 mg; D-pantothenic acid, 10 mg; niacin, 22 mg; menadione sodium bisulfite, 2.33 mg.

[2]Provided as milligrams per kilogram of diet: manganese, 75 from $MnSO_4 \cdot H_2O$; iron, 75 from $FeSO_4 \cdot H_2O$; zinc, 75 mg from ZnO; copper, 5 mg from $CuSO_4 \cdot 5H_2O$; iodine, 75 from ethylene diamine dihydroiodide; selenium, 0.1 from $NaSeO_3$.

Table 4.3. Ingredient composition of experimental Diets 1-5 in Experiment 3.

	Dietary treatments				
	P-deficient control	KH_2PO_4		KH_2PO_4 + Limestone	
Ingredient,%	1	2	3	4	5
Dextrose	16.68	16.61	16.54	16.48	16.28
Cornstarch	33.38	33.22	33.066	33.09	32.80
Soybean meal	42.00	42.00	42.00	42.00	42.00
Spray dried plasma protein	-	-	-	-	-
Meat and bone meal	-	-	-	-	-
Dicalcium phosphate	0.10	0.10	0.10	0.10	0.10
Soybean oil	5.00	5.00	5.00	5.00	5.00
Limestone	1.65	1.65	1.65	1.91	2.18
DL- Methionine	0.30	0.30	0.30	0.30	0.30
Salt	0.40	0.40	0.40	0.40	0.40
Vitamin mix[1]	0.20	0.20	0.20	0.20	0.20

Table 4.3. cont.

Mineral mix[2]	0.15	0.15	0.15	0.15	0.15
Choline chloride	0.10	0.10	0.10	0.10	0.10
KH_2PO_4	-	0.23	0.454	0.23	0.45
Bacitracin-BMD premix[3]	0.04	0.04	0.04	0.04	0.04
Calculated analysis:					
Ca	0.77	0.77	0.77	0.87	0.97
Nonphytate P	0.11	0.16	0.21	0.16	0.21

[1]Provided per kilogram of diet: retinyl acetate, 4,400 IU; cholecalciferol, 25µg; DL-α-tocopheryl acetate, 11 IU; vitamin B_{12}, 0.01 mg; riboflavin, 4.41 mg; D-pantothenic acid, 10 mg; niacin, 22 mg; menadione sodium bisulfite, 2.33 mg.

[2]Provided as milligrams per kilogram of diet: manganese, 75 from $MnSO_4 \cdot H_2O$; iron, 75 from $FeSO_4 \cdot H_2O$; zinc, 75 mg from ZnO; copper, 5 mg from $CuSO_4 \cdot 5H_2O$; iodine, 75 from ethylene diamine dihydroiodide; selenium, 0.1 from $NaSeO_3$.

[3]Contributed 13.75 mg/kg of bacitracin methylene disalicylate (5.5%).

Table 4.4. Ingredient composition of experimental Diets 6-11 in Experiment 3.

	Dietary treatments					
	Soybean meal		Spray dried plasma protein		Meat and bone meal	
Ingredient,%	6	7	8	9	10	11
Dextrose	12.52	8.35	14.18	11.68	16.18	15.68
Cornstarch	25.04	16.71	30.88	28.38	32.88	32.38
Soybean meal	42.00	42.00	42.00	42.00	42.00	42.00
Test soybean meal	12.50	25.00	-	-	-	-
Spray dried plasma protein	-	-	5.00	10.00	-	-
Meat and bone meal	-	-	-	-	1.00	2.00
Dicalcium phosphate	0.10	0.10	0.10	0.10	0.10	0.10
Soybean oil	5.00	5.00	5.00	5.00	5.00	5.00
Limestone	1.65	1.65	1.65	1.65	1.65	1.65
DL-Methionine	0.30	0.30	0.30	0.30	0.30	0.30
Salt	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin mix[1]	0.20	0.20	0.20	0.20	0.20	0.20

Table 4.4. cont.

Mineral mix[2]	0.15	0.15	0.15	0.15	0.15	0.15
Choline chloride	0.10	0.10	0.10	0.10	0.10	0.10
KH_2PO_4	-	-	-	-	-	-
Bacitracin-BMD premix[3]	0.04	0.04	0.04	0.04	0.04	0.04
Calculated analysis:						
Ca	0.80	0.84	0.77	0.78	0.87	0.97
Nonphytate P	0.18	0.26	0.16	0.21	0.16	0.21

[1]Provided per kilogram of diet: retinyl acetate, 4,400 IU; cholecalciferol, 25µg; DL-α-tocopheryl acetate, 11 IU; vitamin B_{12}, 0.01 mg; riboflavin, 4.41 mg; D-pantothenic acid, 10 mg; niacin, 22 mg; menadione sodium bisulfite, 2.33 mg.

[2]Provided as milligrams per kilogram of diet: manganese, 75 from $MnSO_4 \cdot H_2O$; iron, 75 from $FeSO_4 \cdot H_2O$; zinc, 75 mg from ZnO; copper, 5 mg from $CuSO_4 \cdot 5H_2O$; iodine, 75 from ethylene diamine dihydroiodide; selenium, 0.1 from $NaSeO_3$.

[3]Contributed 13.75 mg/kg of bacitracin methylene disalicylate (5.5%).

Table 4.5. Analyzed nutrient composition of ingredients used in Experiments 1, 2, and 3.

Item	Soybean meal	Spray dried plasma protein	Meat and bone meal
DM[1],%	90.1	91.5	93.1
CP[1],%	50.26	79.00	46.63
CF[1],%	0.02	0.03	11.98
Crude Fiber,%	2.70	0.28	2.19
Ash[2],%	6.21	6.33	30.70
Ca[1],%	0.28	0.10	9.93
Total P[1],%	0.59	0.98	4.89

[1]DM = dry matter; CP = crude protein; CF = crude fat; Ca = calcium; P = phosphorus.

Table 4.6. Apparent ileal P digestibility values determined in precision-fed chicks in Experiment 1.[1]

Dietary treatment	NPP[1] intake (mg)	Ileal P digestibility[2] (%)
1. 8 g soybean meal	17.6	64[b]
2. 8 g meat and bone meal	391.2	42[c]
3. 8 g spray dried plasma protein	78.4	94[a]
Pooled SEM		3.5

[a-c] Means within a column with no common superscript differ significantly ($P<0.05$).

[1]NPP indicates non-phytate P.

[2]Values are means of four pens of four chicks at 21 days of age.

Table 4.7. Ileal P digestibility and excreta P retention values for chicks in Experiment 2.[1]

Dietary treatment	Total dietary P (%)	Ileal P digestibility (%)	Excreta P retention (%)
1. 18% soybean meal	0.100	74[de]	49[d]
2. 36% soybean meal	0.311	85[bc]	63[c]
3. 54% soybean meal	0.391	84[c]	61[c]
4. 7% spray dried plasma protein	0.082	80[cd]	74[b]
5. 14% spray dried plasma protein	0.180	94[ab]	93[a]
6. 21% spray dried plasma protein	0.241	94[a]	90[a]
7. 2.0% meat and bone meal	0.093	79[cd]	35[e]
8. 4.0% meat and bone meal	0.190	66[ef]	39[e]
9. 6.0% meat and bone meal	0.312	66[e]	32[e]
10. 0.0% meat and bone meal	0.302	83[cd]	64[c]
11. 2.0% meat and bone meal	0.420	65[f]	64[c]
12. 4.0% meat and bone meal	0.495	60[f]	59[c]
Pooled SEM		3.2	2.6

[a-f] Means within a column with no common superscript differ significantly ($P<0.05$).

[1]Values are means of five pens of five chicks at 21 days of age.

Table 4.8. Linear relationship between ileal or excreta P outputs vs. total dietary P content in Experiment 2.

Item	Regression equation[1]	SE[2] of the slope	SE[2] of the intercept	R^2	Digestibility/ retention coefficient
Soybean meal					
True ileal P digestibility	Y= 0.167X + 0.086	0.03	0.07	0.69	0.833
True excreta P retention	Y= 0.486X + 0.024	0.05	0.10	0.89	0.514
Spray dried plasma protein					
True ileal P digestibility	Y= 0.024X + 0.077	0.02	0.04	0.08	0.976
True excreta P retention	Y= 0.013X + 0.173	0.03	0.05	0.02	0.987
Meat and bone meal[3]					
True ileal P digestibility	Y= 0.388X – 0.140	0.06	0.13	0.77	0.612
True excreta P retention	Y= 0.675X – 0.018	0.05	0.12	0.93	0.325
Meat and bone meal[4]					
True ileal P digestibility	Y= 0.767X + 1.778	0.05	0.22	0.94	0.233
True excreta P retention	Y= 0.473X – 0.378	0.05	0.20	0.88	0.527

[1]Regression of ileal digesta or excreta P output (g/kg dry matter intake) on dietary P content (g/kg) determined by feeding diets containing graded levels of either soybean meal, spray dried plasma protein 2 or meat and bone meal. The slope represents true P indigestibility. The digestibility and excreta retention coefficients were calculated by subtracting the slope values from one.

[2]SE= Standard error.

[3]Determined using Diets 7-9 in Table 4.2 where the MBM was the only source of dietary P.

[4]Determined using Diets 10-12 in Table 4.2 where the MBM was added to corn-soybean meal-potato protein diet.

Table 4.9. Growth performance and tibia ash content of chicks in Experiment 3.[1]

Dietary treatment	Weight gain (g/chick)	Feed Intake (g/chick)	Gain:feed (g/kg)	Bone ash[3] (mg/tibia)
1. P deficient dextrose-corn starch 0.11% NPP	263^{fg}	409^{e}	644^{cde}	296^{g}
2. As 1 + 0.05% KH_2PO_4	290de	447^{bcd}	651^{bcd}	360de
3. As 1 + 0.10% KH_2PO_4	313^{bc}	486^{a}	644^{cde}	440^{b}
4. As 2 + 0.10% Ca from limestone[2]	279^{efg}	443^{bcde}	635^{cde}	356^{def}
5. As 3 + 0.20% Ca from limestone[2]	299^{cd}	459^{abc}	650^{bcd}	405^{c}
6. As 1 + 12.5% SBM[2]	278^{efg}	419de	663^{bc}	343^{ef}
7. As 1 + 25% SBM[2]	265^{fg}	430^{bcde}	609^{e}	360de
8. As 1 + 5.0% SDPP[2]	318^{b}	463^{ab}	686^{b}	406^{c}
9. As 1 + 10.0% SDPP[2]	353^{a}	486^{a}	726^{a}	507^{a}
10. As 1 + 1.0% MBM[2]	261^{g}	428^{cde}	612de	331^{f}
11. As 1 + 2.0% MBM[2]	281^{def}	428^{bcde}	656^{bc}	377^{d}
Pooled SEM	6.7	12.3	13.7	9.7

[a-g]Means within a column with no common superscript differ significantly (P<0.05).

[1]Values are means of five pens consisting of five chicks.

[2]NPP= nonphytate P, SBM = soybean meal, SDPP= spray dried plasma protein, MBM= meat and bone meal.

[3]Multiple regression of tibia ash (Y; mg) on supplemental P intake (g) from KH_2PO_4 (X_1), KH_2PO_4 + Ca (X_2), SBM (X_3), SDPP (X_4), and MBM (X_5) yielded the equation: $Y = 299.9 + 285.5 \pm 22.1X_1 \pm 234.5 \pm 23.2X_2 \pm 103.9 \pm 16.8X_3 \pm 357.4 \pm 18.2X_4 \pm 177.1 \pm 25.3X_5$ (R^2=0.89). The (±) values are standard errors of the regression coefficients.

Table 4.10. Relative bioavailability of the P in soybean meal, spray dried plasma protein, and meat and bone meal in Experiment 3.

	Total P (%)	Bioavailability values[1](%)	Bioavailable content[2] (%)
Sample		Tibia ash (mg)	Tibia ash (mg)
Soybean meal	0.59	36.4[c]	0.21
Spray dried plasma protein 2	0.98	125.0[a]	1.22
Meat and bone meal	4.89	75.5[b]	3.69

[a-c]Values within the column containing no common superscripts are significantly different ($P < 0.05$) as determined using the regression coefficients and standard errors in the multiple regression equation in footnote 2 of Table 4.9.

[1]Calculated by the slope-ratio method using the multiple regression equation in footnote 3 of Table 4.9. Bioavailability values are relative to the P in KH_2PO_4 which was set at 100% for soybean meal and spray dried plasma protein. The value for meat and bone meal is relative to KH_2PO_4 + Ca (X_2) in the multiple regression equation.

[2]Bioavailable content=total P × bioavailability value.

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CHAPTER 5

CONCLUSIONS

The focus of the research in this thesis was to evaluate the bioavailability and digestibility of P in SBM, SDPP and MBM using several different methods. The experiments in Chapter 3 determined P bioavailability via excreta P retention values using the precision-fed rooster assay. The experiments in Chapter 4 were conducted to determine P bioavailability using a precision-fed chick assay, an ad-libiltum fed chick assay and a chick bone ash bioassay. There are few published articles presenting P bioavailability values determined in chicks using the bone ash bioassay for SBM and MBM.

Results of experiments conducted in Chapter 3 indicated that the precision-fed rooster assay may be useful when estimating P availability of feed ingredients if the NPP or available P intake is low. When the NPP intake exceeded 76 mg, excess P may be excreted in the urine. Due to the need to have a low NPP intake, the precision-fed rooster assay may not be the most useful or practical method to estimate P bioavailability in feed ingredients for poultry.

Experiments conducted in Chapter 4 yielded results that often contradicted one another. Ileal P digestibility and excreta P retention values varied within SBM and MBM among methods. The ad-libitum chick assay generally yielded higher P digestibility values for SBM than MBM, whereas the bone ash bioassay yielded higher P bioavailability values for MBM than SBM. It is unknown why this discrepancy was observed, but it was concluded that more research among these bioassays must be done. The P digestibility or bioavailability for SDPP was high among all methods, suggesting it is a highly digestible feed ingredient for P.